IN VIVO DETERMINATION OF METABOLIC FUNCTION FOR USE IN THERAPY MANAGEMENT

CROSS REFERENCE TO RELATED APPLICATIONS

Not Applicable

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

Not Applicable

BACKGROUND OF THE INVENTION

The economic and social burden to society of managing a number of costly human medical problems, including digestive disorders, cancer, critical care, infectious diseases, atherosclerosis and neurodegenerative disorders, is severe. Historically, clinicians have tried different ways to assess the status of a patient and monitor the effectiveness of therapy. For example, it has been recognized since antiquity that the monitoring of breath is desirable, as it contains clues to many diseases and metabolic processes in the body.

Breath tests are useful, specifically, as non-invasive procedures for the detection of isotopically labelled tracer substrates, particularly the stable carbon isotope ^{13}C. Breath test tracer substrates may be given orally, no blood need be drawn and samples may be collected easily.

Historically, tracers have been used in diverse scientific settings to follow the metabolic fate of tracer- labelled molecules in dynamic systems, e.g., to determine rates of synthesis, transformation or degradation of molecules in vivo, in intact organisms or perfused organs, or in vitro, with tissue homogenates or subcellular fractions.

The most commonly used tracers are radioactive, e.g., ^{3}H, 14C or ^{32}P-labelled molecules. These can be "traced" by measuring the intensity and location of the radiation emanating from the tracers as a function of time. Non-radioactive nuclides, or stable isotopes such as ^{13}C can also be used advantageously as tracers, especially in metabolic studies. Stable isotope tracers can be "traced" by examining the properties of their molecular mass as it becomes diluted over time by the natural abundance masses also contained within the biological matrix under study in the tracer experiment.

The most frequent approach for using tracers is to incorporate a desired nuclide atom into a target molecule whose transformation is to be studied as a function of time, and then to follow the metabolic fate of the molecule as it undergoes one or more biological interconversions. Another approach, used more in determining properties of enzyme systems, focuses on determining the rate at which the nuclide disappears from the tracer labelled molecule and then reappears after incorporation into biological variants of the initial molecule, e.g., metabolites.

The analysis of nuclide labeling patterns and the quantitation of tracer rates of appearance and disappearance are often time consuming and technically complex operations. For example, while enzymology in vitro can be extensively manipulated so as to minimize the confounding effects of biochemical recycling and of metabolic integration on the calculation of pertinent kinetic parameters, far fewer possibilities exist for similar manipulations in vivo.

Another drawback in the historic application of labelled tracer probes is that easy, non-invasive determinations such as breath tests are often not possible, and, as such, invasive

methods like biopsy may need to be used. Although breath tests have been shown to be useful in conjunction with determinations of hepatic function and enzyme induction, gastric emptying, maldigestion/malabsorption, and intermediary metabolism, one notable disadvantage or limitation of the breath test for disease diagnosis is that while the labelled end product can be measured, e.g., $^{13}CO_2$, this does not provide information on various pools and fluxes the labelled substrate and its metabolites pass through, in order to give an indication of the presence or absence of a disease condition.

It would, therefore, be beneficial to the art of utilizing tracers in therapy management to systematize and streamline their design and application, especially for the purpose of determining the status of processes critical to the maintenance of normal function in the context of health and disease in vivo, without the drawbacks mentioned above.

SUMMARY OF THE INVENTION

It is an object of the present invention to provide in vivo analytical methods that allow for diagnosis and management of therapy for diseases involving discrete biochemical pathways. In the method of the invention, a labelled tracer probe, a specifically designed substrate of a "gateway" enzyme, an enzyme marking a regulatory point in a discrete biochemical pathway, is administered to a subject; a labelled product of the action of the enzyme is measured; and the appearance and concentration of the product are related to the disease condition of interest. Determination of the rate of substrate-product conversion of the gateway enzyme allows for the analysis to be made. The method involves administering a defined amount of a labelled "metaprobe" substrate of the gateway enzyme to a subject, at a site that provides access to a desired pool of the gateway enzyme in the subject, and measuring the amount of the chosen labelled product. The presence and amount of the chosen labelled product in, e.g., the breath, definitively indicates that the labelled metaprobe has been metabolized by the specific enzyme in the specific biochemical pathway under consideration, and from the calculated rate of substrate- product conversion by the gateway enzyme, the desired diagnostic determination may be made. Likewise, if a specific therapeutic treatment for a disease is underway with a patient, this method allows for a minimally invasive assessment of the effectiveness of the treatment, with minimal discomfort to the patient.

The usefulness of the method of the invention depends on careful determination of the appropriate metaprobe substrate of the selected gateway enzyme. One aspect of such a determination can consist of structurally modifying an enzyme's natural substrate into a surrogate substrate whose metabolism can be measured in vivo in spite of confounding biochemical and physiological circumstances. Another aspect of metaprobe selection is a determination of the most appropriate location of the labelled portion so that the chosen product to be measured is labelled appropriately. Additionally, a further desirable characteristic of a metaprobe is that its enzymatic conversion product be (1) accessible by non-invasive or minimally invasive means, to allow isolation from the biological system containing it, and then be (2) amenable to rapid quantitative analysis for its isotopic content. In other words, the ideal metaprobe should permit clear and rapid differentiation between precursor and product so that calculations of rate of precursor conversion into product, and other rate-dependent parameters, are unambiguous.

In another aspect, the invention relates to labelled metaprobes, and to methods for their synthesis, for use in